

CLAIMS

What we claim is:

- 5 1. A method for providing an extended linear dynamic range in an
 analyte assay that uses as signals light collected from light scattering
 particles at a plurality of sites, said method comprising:
 - (a) detecting integrated light from the plurality of sites with a sensor using more
10 than one exposure time, such that signals generated by the sensor are linearly proportional
10 to the integrated light intensities detected at one or more of sites with extreme integrated
 light intensities, and
 - (b) combining the signals from at least two of the different exposure times to
 quantify the light from one or more of the sites with extreme integrated light intensities,
15 thereby providing an extended linear dynamic range.
- 15 2. A method for providing an extended dynamic range in an analyte
 assay that uses light collected from light scattering particles, said
 method comprising:
 - (a) detecting integrated light for a single exposure time with a sensor that provides
20 non-destructive reading of signals from individual pixels or groups of pixels;
 - (b) reading the signals from pixels at time intervals such that any pixel that
 approaches saturation is reset; and
 - (c) combining the signals read at said time intervals for each reset pixel to quantify
 the detected integrated light, thereby providing the extended dynamic range.
- 25 3. A method for providing an extended linear dynamic range in an analyte
 assay that uses light from light scattering particles as signals, said
 method comprising:
 - (a) detecting integrated light with a sensor, wherein the intensity of integrated light
30 from one or more assay sites is reduced by one or more light filters to an extent

that the signal generated by the sensor is linearly proportional to the integrated light detected; and

(b) scaling the signal by factors determined by the light transmitted by said one or more filters to quantify the integrated light from one or more of the sites, thereby providing the extended dynamic range.

4. A method for providing an extended linear dynamic range in an analyte assay that uses light collected from light scattering particles as signals, said method comprising:

(a) detecting integrated light intensities from a plurality of sites with a sensor;
(b) repeating detection using one or more light filters such that signals generated by the sensor are linearly proportional to the integrated light detected at one or more of sites with high integrated light intensities; and

(c) scaling the signals from said one or more sites by factors based on the light transmitted by said one or more filters to quantify the integrated light from one or more of the sites, thereby providing the extended dynamic range.

5. A method for providing an extended linear dynamic range in an analyte assay that uses light collected from light scattering particles at a plurality of sites as signals, said method comprising:

(a) detecting integrated light from the plurality of sites with a sensor;
(b) counting the number of particles at one or more sites with low integrated light intensities, wherein signals generated by the sensor are not linearly proportional to the integrated light detected at one or more of the sites with low integrated light intensities; and

(c) normalizing signals from integrated light intensities with the numbers of particles at the site for the one or more sites with low integrated light intensities, thereby providing the extended linear dynamic range.

6. A method for providing an extended dynamic range in an analyte assay that uses light collected from light scattering particles at a site as signals, wherein light collected from said light scattering particles is not

proportional to the number of particles at the site, said method comprising:

(a) detecting integrated light with a sensor;

5 (b) generating a standard curve of integrated light intensities versus the number of light scattering particles; and

(c) applying the standard curve to calculate the number of particles at the site based on the detected integrated light, thereby providing the extended dynamic range of the analyte assay.

10 7. The method of claim 1, wherein step (b) comprises scaling the signals by a conversion factor based on the exposure times.

15 8. The method of claim 1, wherein the exposure times are selected from the group consisting of at least 10 milliseconds, 100 milliseconds, 1 second, 10 seconds, 1000 seconds, and 3600 seconds.

9. The method of claim 1, wherein a total of at least two, three, four, five, or six different exposure times are used to detect integrated light.

20 10. The method of claim 2, wherein the integrated light detected by the individual pixel or groups of pixels are from one or more sites of the assay.

25 11. The method of claim 2 wherein step (c) comprises summing the signals read at said time intervals, or averaging the signals read at said time intervals.

12. The method of claim 2, wherein individual pixels or group of pixels are reset at registered time intervals.

30 13. The method of claim 2, wherein individual pixels or group of pixels can be accessed randomly or separately reset.

14. The method of claim 2, wherein the time interval for resetting individual pixels or group of pixels is selected to maintain the signal generated by the pixels within the linear response range of the sensor.

5 15. The method of claim 2, wherein the sensor is a charged injection device.

16. The method of claim 3 or 4, wherein the light transmitted by the filters is measured using a white light source.

10 17. The method of claim 3 or 4, wherein the factors for scaling the signal are calculated from the transmission curve for the filter.

18. The method of claim 3 or 4, wherein scaling the signal comprises the steps of:

- 15 (a) dividing the of the assay by the wavelength dependent transmission curve of the one or more filters used to collect the image;
- (b) setting to zero the values from pixels that were saturated; and
- (c) combining the two or more signals.

20 19. The method of claim 3 or 4, wherein the one or more filters are selected from the group consisting of longpass filters, shortpass filters, bandpass interference filters, filter wheels, neutral density filters, color filters, notch filters, super notch filters, supernotch plus filters, and filter monochrometers.

25 20. The method of claim 3 or 4, wherein the amount of light transmitted by the one or more filters is selected from the group consisting of 1%, 3.2%, 6.3%, 10%, 13%, 16%, 20%, 25%, 32%, 40%, 50%, 63%, 70%, and 80% of the light entering the filter.

21. The method of claim 6, wherein the particles are counted by increasing the magnification of the one or more sites.

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22. The method of claim 6, wherein the particles are counted in more than one fields, and the counted fields constitute a statistically significant portion of one or more sites.

23. The method of claim 6, wherein the standard curve is generated from a dilution series comprising sites with different numbers of particles per unit area or unit volume.

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24. The method of claim 23, wherein the sites comprising the dilution series are all associated with the same physical form of the analyte assay.

25. The method of claim 6, wherein the standard curve is generated semi-analytically from fits to one or more deconvolution equations.

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26. The method of claim 3, wherein the analyte assay uses a plurality of assay sites.

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27. The method of claims 1, 2, 3, 4, 5, or 6, wherein the light from the light scattering particles comprise light scattered by the light scattering particles, light emitted by fluorescent entities on the light scattering particles, or both.

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28. The method of claim 1, wherein the extreme integrated light intensities at one of the sites is high integrated light intensities, low integrated light intensities, or both where more than one signal are detected from the site.

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29. The method of claim 1, 2, 3, 4, 5, or 6, wherein the extended dynamic range comprises integrated light intensities quantified over at least four, five, six, or seven orders of magnitude.

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30. The method of claim 1, 2, 3, 4, 5, or 6, wherein the dynamic range is extended by at least one order of magnitude over the dynamic range of an assay without the extension of dynamic range and the extended dynamic range is linear.

31. The method of claim 1, 2, 3, 4, 5, or 6, which further comprises forming an image of one or more of the sites with the combined signals.

32. The method of claim 31, wherein the formation of the image comprises the steps of identifying background portions of the image, and removing signals corresponding to the background portions of the image.

5 33. The method of claims 1, 2, 3, 4, 5, or 6, wherein the sensor is selected from the group consisting of a camera, a photographic film, a video camera, a charged-coupled device, a charged injection device, a photodiode, a photodiode array, and a photomultiplier tube.

10 34. The method of claim 1, 2, 3, 4, 5, or 6, wherein said plurality of sites are separately addressable sites.

15 35. The method of claims 1, 2, 3, 4, 5, or 6, wherein said plurality of sites are associated with a physical form selected from the group consisting of a slide, a membrane, a filter, a test tube, a vial, a microtiter plate, a microarray, a small volume device, or a gel.

20 36. The method of claim 1, 2, 3, 4, 5, or 6, wherein said plurality of sites are present in a sample selected from the group consisting of a tissue, a tissue section, a cell culture, a cell, a cell organelle, a chromosome preparation, and a chromosome.

37. The method of claim 1, wherein step (b) comprises removing signals corresponding to the background portion of the spectrum of light detected.